

HELLER EHRMAN WHITE & McAULIFFE LLP

Sheet 1 of 23 Applicant: Ault-Riche et al. DKT. No. 25885-1751

Priority claimed to 60/219,183
For:COLLECTIONS OF BINDING PROTEINS AND
TAGS AND USES THEREOF FOR NESTED SORTING
AND HIGH THROUGHPUT SCREENING

Sorting by pools

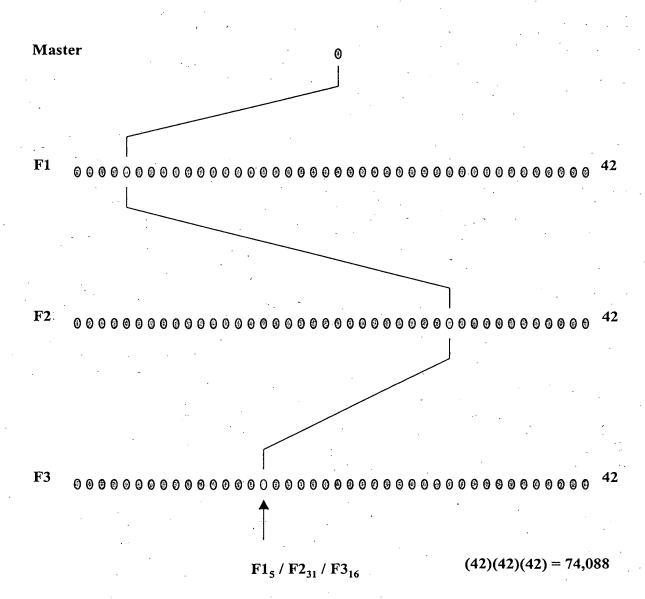


FIGURE 1

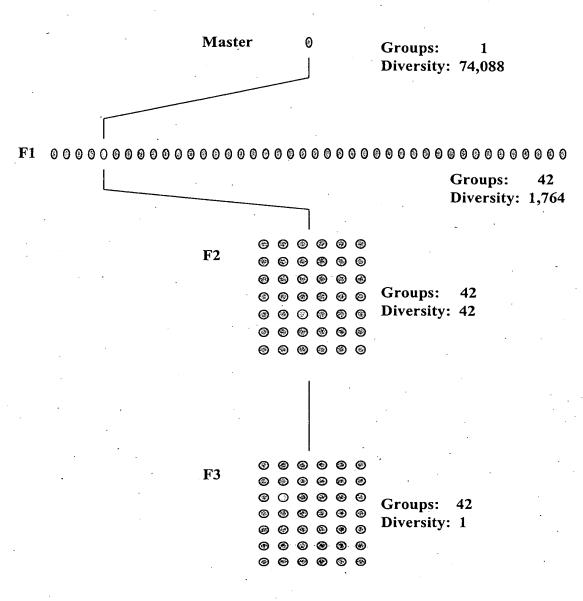
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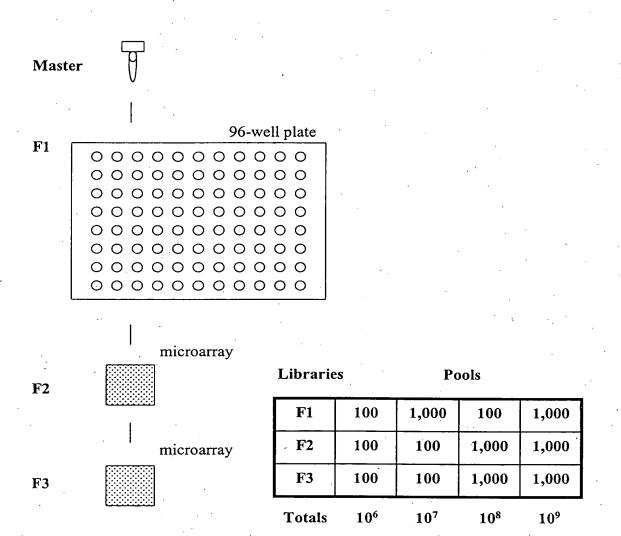
Sorting by pools: Decreasing pool diversities





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Sorting by pools: Screening large diversity libraries



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Searching a mutation library

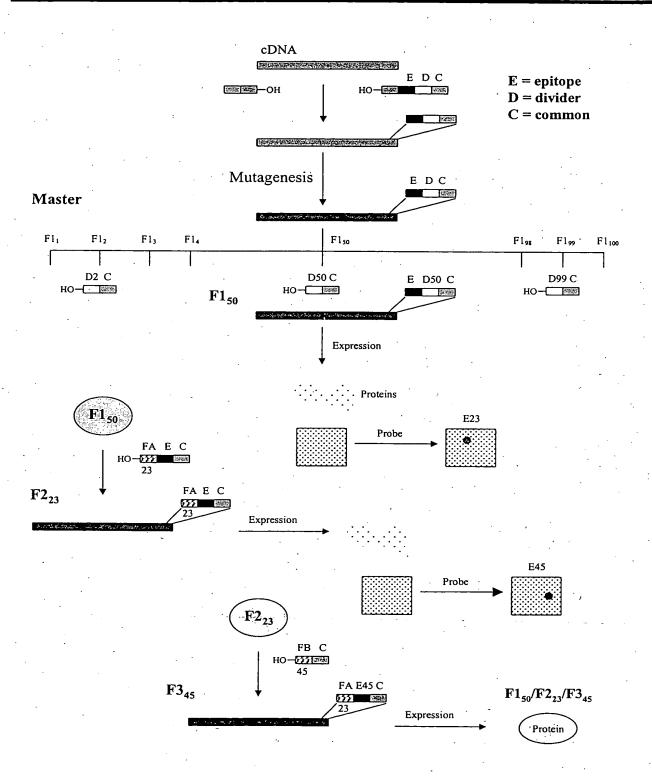


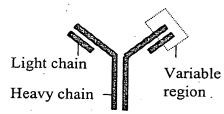
FIGURE 4

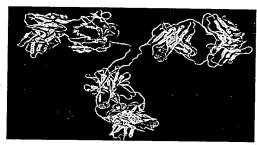
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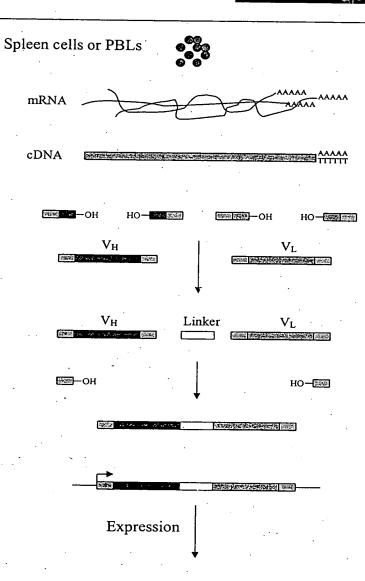
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Making a recombinant antibody library

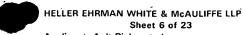
Basic antibody structure





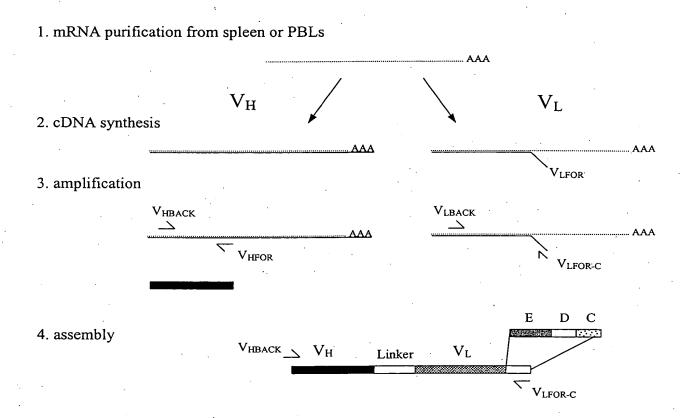


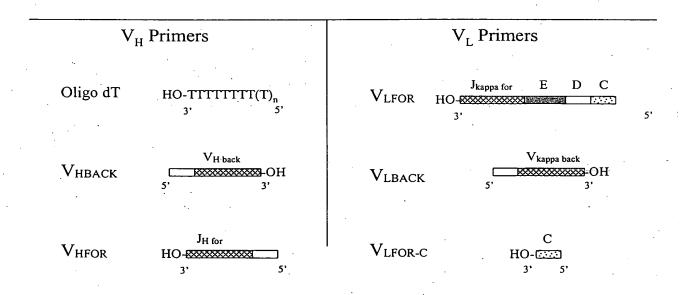
Antibodies

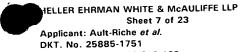


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Creating the master antibody library: Primer incorporation

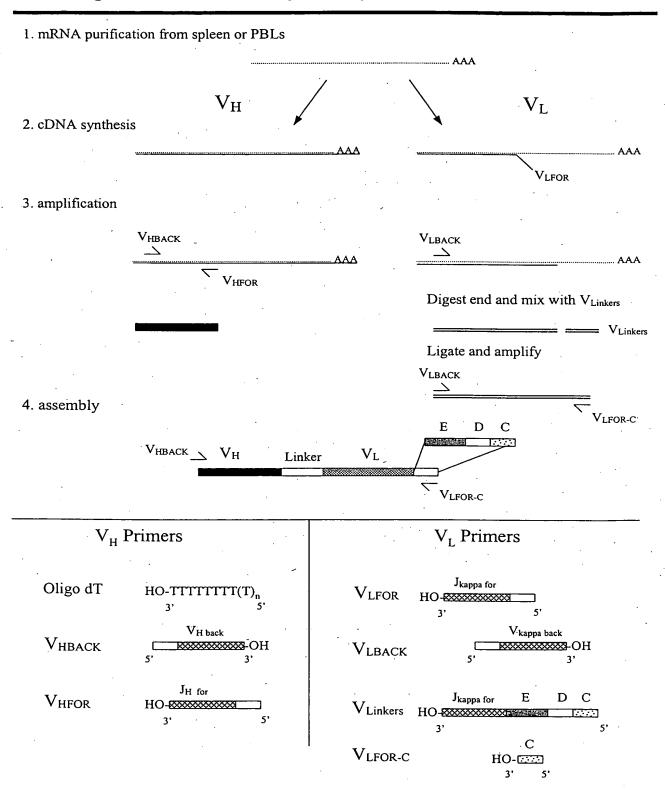






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Creating the master antibody library: Linker addition





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Searching a recombinant antibody library

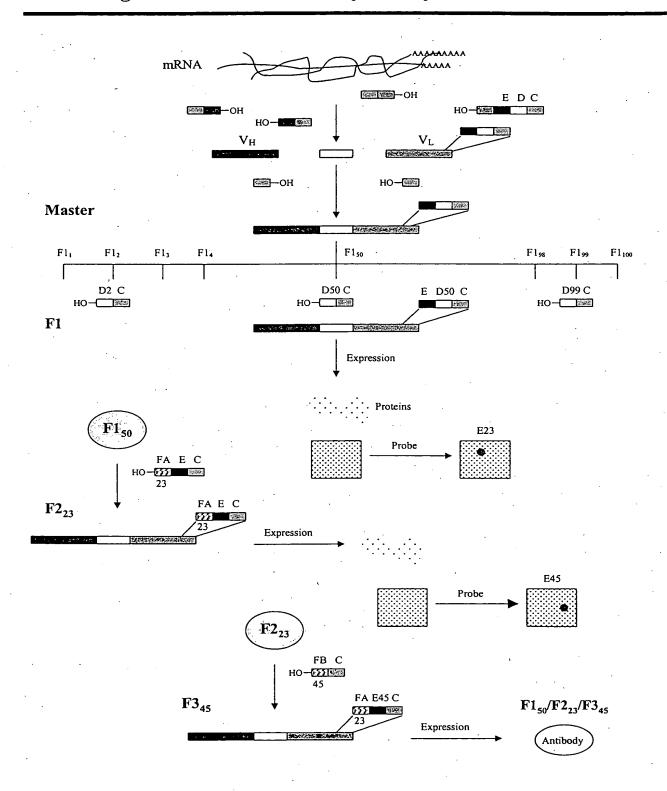
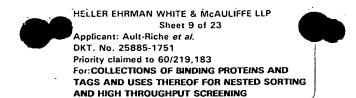


FIGURE 8



Physical elements to include in the kits and combinations

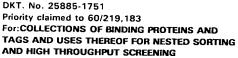
- Anti-tag Arrays™
- Primer sets

E D C
HO- SOME
$$D_1 - D_{1,000}$$

FA E C
HO- FB C

- Readers
- Software







Making the V_{LFOR} primers: Solid phase synthesis

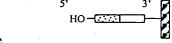
J_{kappa for} E D C
VLFOR: HO

3'

S'

J_{kappa for} Epitope D Common

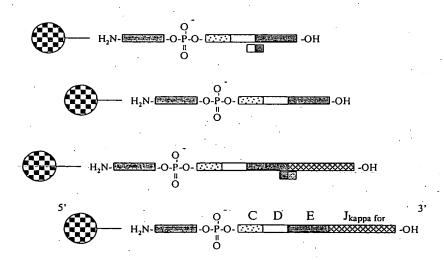
- Jkappa for Epitope D Comm
- 1. Synthesize oligo on solid support



2. Add aminolink prior to cleavage

3. Couple to tosyl activated magnetic beads

4. Extend by hybridizing with DNA patch and ligating



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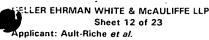
DKT. No. 25885-1751
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Making the V_{LFOR} primers: Overlapping hybridization

	VLFOR: HO-************************************
	J _{kappa for} Epitope D Common
•	Synthesize 4,028 different oligos: (26 for J _{kappa for} , 2,000 for Epitope; 2,000 for D; 2 for Common)
2.	Assemble oligos for + and - strands of the different regions
	но
3.	Ligase the assembled oligos
4.	lst strand synthesis with biotinylated primer
	жон
•	2 nd strand synthesis with non-biotinylated primer *OH
6.	Bind to avidin coated magnetic beads and then denature
	у кон
7.	Purify non-biotinylated ssDNA
	J _{kappa for} Epitope D Common



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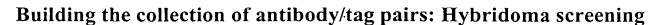
DKT. No. 25885-1751

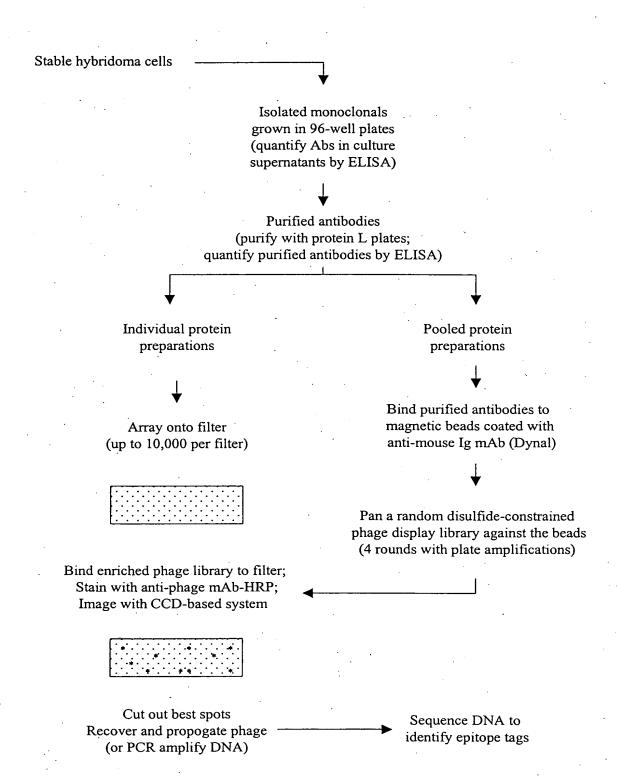
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TABLE 3 Primers for PCR Amplification of Human Antibody Variable Regions (V genes)

1. V gene primary PCR

A. Human VH buck primers (sense)

Huvhiaback 5'-Cag GTG Cag CTG GTG Cag TCT GG-3'
Huvhaback 5'-Cag GTC AAC TTA AGG GAG TCT GG-3'
Huvhaback 5'-Cag GTG Cag CTG GTG GAG TCT GG-3'
Huvhaback 5'-Cag GTG Cag CTG Cag GAG TCG GG-3'
Huvhaback 5'-Gag GTG Cag CTG TTG Cag TCT GC-3'
Huvhaback 5'-Cag GTA Cag CTG CAG CAG TCA GG-3'

B. Human'IH forward primers (anti-sense)

Huiht-2FOR 5'-TGA GGA GAC GGT GAC CAG GGT GCC-3'
Huiht3FOR 5'-TGA AGA GAC GGT GAC CAT TGT CCC-3'
Huiht4-5FOR 5'-TGA GGA GAC GGT GAC CAG GGT TCC-3'
5'-TGA GGA GAC GGT GAC CGT GGT CCC-3'

C. Human V kappa back primers (sense)

HuVklaBACK
HuVk2aBACK
HuVk3aBACK
HuVk3aBACK
HuVk4aBACK
HuVk5aBACK
HuVk5aBACK
HuVk6aBACK
HuVk6aBACK
HuVk6aBACK
HuVk6aBACK
HuVk6aBACK
HuVk6aBACK
HuVk6aBACK
HuVk6aBACK
HuVk6aBACK

S'-GAC ATC GTG ATC ACG CAG TCT CC-3'

S'-GAC ATT GTG CTG ACT CAG TCT CC-3'

C. Human V lambda back primers (sense)

HuV\1BACK
HuV\2BACK
HuV\2BACK
HuV\3aBACK
HuV\3aBACK
HuV\3bBACK
HuV\3bBACK
HuV\3BACK
HuV\3BACK
HuV\3BACK
HuV\3BACK
HuV\3BACK
HuV\3BACK
HuV\3BACK
HuV\3BACK
HuV\3BACK
S'-CAC GTT ATA CTG ACT CAG CCG CC-3'
HuV\3BACK
HuV\3BACK
S'-CAG GCT GTG CTC ACT CAG CCC CC-3'
HuV\3BACK
S'-AAT TTT ATG CTG ACT CAG CCC CA-3'

D. Human I kappa forward primers (anti-sense)

HUJKIFOR 5'-ACG TIT GAT TTC CAC CTT GGT CCC-3'
HUJK2FOR 5'-ACG TIT GAT CTC CAG CIT GGT CCC-3'
HUJK3FOR 5'-ACG TTT GAT ATC CAC CTT GGT CCC-3'
HUJK4FOR 5'-ACG TTT GAT CTC CAC CTT GGT CCC-3'
HUJK5FOR 5'-ACG TTT AAT CTC CAG TCG TGT CCC-3'

D. Human I lambda forward primers (anti-sense)

Hu JALFOR: --- 5'-ACC TAG GAC GGT GAC CTT GGT CCC-3'
Hu JA2-3FOR 5'-ACC TAG GAC GGT CAG CTT GGT CCC-3'
Hu JA4-5FOR 5'-ACC TAA AAC GGT GAG CTG GGT CCC-3'

continues

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FIGURE 13B

TABLE J Contin	u₄d FIGURE 13B							
2. Linker fragmer	nt PCR							
-	seUH for scFv linker (sonse)							
	FR4 heavy							
	RHuihi-2 5'-GC ACC CTG GTC ACC GTC TUU TCA GGT GG-3'							
	RHuJH3 5'-GG ACA ATG GTC ACC UTC TCT TCA GGT GO-3'							
	RHuJH4-5 5'-GA ACC CTG GTC ACC GTC TCC TCA GGT GG-3' RHuJH6 5'-GG ACC ACG GTC ACC GTC TCA GGT GG-3'							
	RIMIN 5 FOR ACC ACC STC ACC ACC STC ACC ACC ACC ACC ACC ACC ACC ACC ACC A							
F Panaro	eVk for scFv linker (anti-sonse)							
7. Mergan								
	RHUVKIBBACKFV 5'-GG AGA CTG GGT CAT CTG GAT GTC CGA TCC GCC-3'							
	RHuVk2aBACKFv 5'-OG AGA CTG AGT CAT CAC AAC ATC CGA TCC GCC-3'							
	RHuVk3aBACKFy 5'-GG AGA CTG CGT CAA CAC AAT TTC CGA TCC GCC-3'							
•	RHUVk4uBACKFv 5'-GG AGA CTG GGT CAT CAC GAT GTC CGA TCC GCC-3' RHUVk5uBACKFv 5'-GG AGA CTG CGT GAG TGT CGT TTC CGA TCC GCC-3'							
	RHIIVK61BACKFV 5'-GG AGA CTG AGT CAG CAC AAT TTC CGA TCC GCC-3'							
P. Reverse VA for scFv linker (anti-sense)								
	FRI lightlinker							
	BACKIEV 5'-GG CGG CTG CGT CAA CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'							
	BACK2Fv 5'-GC AGG CTG AGT CAG AGC AGA CTG CGA TCC GCC ACC GCC AGA G-3' BACK3aFy 5'-GG TGG CTG AGT CAG CAC ATA GGA CGA TCC GCC ACC GCC AGA G-3'							
	BACK3aFv 5'-GG TGG CTG AGT CAG CAC ATA GGA CGA TCC GCC ACC GCC AGA G-3' BACK3bFv 5'-GG GTC CTG AGT CAG CTC AGA AGA CGA TCC GCC ACC GCC AGA G-3'							
	BACK4FV 5'-GG CGG TTG AGT CAG TAT AAC GTG CGA TCC GCC ACC GCC AGA G-3'							
RHu∨λ	BACKSFV 5'-GA COO CTG AOT CAG CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'							
RHuVλ	BACK6PV 5'-TG GGG CTG AGT CAG CAT AAA ATT COA TCC GCC ACC GCC AGA G-3'							
;	mers for introduction of restriction sites"							
į	VII buck (Sfi) primers (sense)							
HuVHlal								
HuVH2a	TC GCA ACT GC <u>G GCC</u> CAG CC <u>G GCC</u> ATG GCC CAG UTG CAG CTG GTG CAG TCT GG-3'							
ſ	TC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTC AAC TTA AGG GAG TCT GG-3'							
HuVH3aB								
5'-GTC C HuVH4aB	TC GCA ACT GC <u>G GCC</u> CAG CC <u>G GCC</u> A'IG GCC GAG GTG CAG CTG GTG GAG TCT GG-3'							
	TC GCA ACT GC <u>G GCC</u> CAG CC <u>G GCC</u> ATG GCC CAG GTG CAG CTG CAG GAG TCG GG-3'							
HuVH5aB	ACKSfi							
	TC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG TTG CAG TCT GC-3'							
HuVH6uB	TO GOA ACT GO <u>G GOO CAG COO GOO</u> ATO GOO CAG GTA CAG CTO CAG CAG TOA GO-3'							
	To do we had and de data in a second antenders and fendally							
II. Human	I kappa forward (Not) primers (anti-sense)							
. 1	IuJk 1 FORNot							
5	'-GAG TCA TTC TCG ACT T <u>GC GGC CGC</u> ACG TTT GAT TTC CAC CTT GGT CCC-3'							
	IUIKZFORNOI							
	'-OAG TCA TI'C TCG ACT T <u>OC GGC CGC</u> ACG ITT GAT CTC CAG CTT GGT CCC-3'							
',	H. Human J kappa jorward (Not) primers (anti-sense) (Continued)							
	1uJk3PORNot —FR4 ight————							
	S'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT ATC CAC TTT GGT CCC-3							
	Hulk4FORNot							
	I'-GAG TCA TTC TCG ACT T <u>GC GGC CGC</u> ACG TTT GAT CTC CAC CTT GGT CCC-3 ⁻ Iu <i>lis</i> FORNot							
	'-GAG TCA TTC TCG ACT T <u>GC GGC CGC</u> ACG TTT AAT CTC CAG TCG TGT CCC-3'							
•								
· · · · · · · · · · · · · · · · · · ·	l. Human I lambdo forward (Not) primers (anti-sense)							
	iu Л1FORNOT ————————————————————————————————————							
	'-QAG TCA TTC TCG ACT T <u>GC GGC CGC</u> ACC TAG GAC GGT GAC CTT GGT CCC-3'							
	IU JI2-3FORNOT '-GAG TCA TTC TCG ACT T <u>GC GGC CGC</u> ACC TAG GAC GGT CAG CTT GGT CCC-3'							
	in 114-5FORNOT							
5'	'-GAO TCA TTC TCG ACT T <u>GC GGC CQC</u> ACC TAA AAC GGT GAG CTG GGT CCC-3'							

^{*}Recugnition site for restriction enzyme is underlined.

Tag and assemble immunoglobulin genes

 $\downarrow \qquad \qquad (V_{H}\text{-linker-}V_{L})$ $\blacksquare \text{ tags}$

Create 1,000 sub-libraries by separate PCR amplification reactions using tag-specific PCR primers

1,000 sub-libraries

. 3

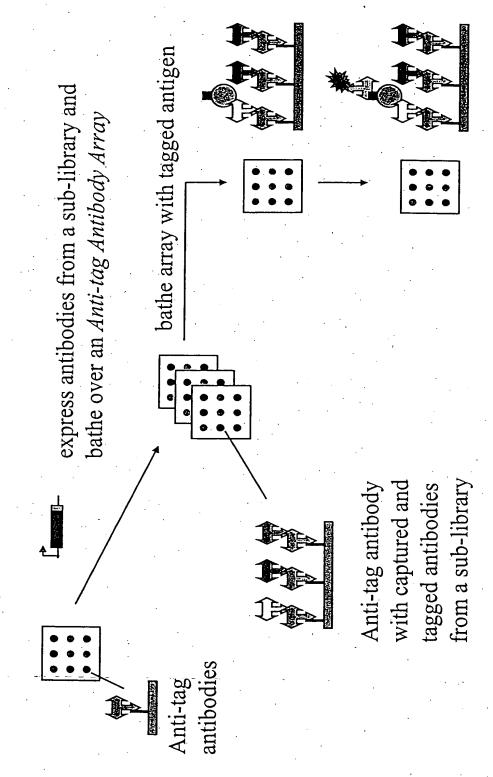
FIGURE 14A

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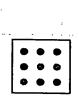


ID spot containing the antigen with a labeled developing Ab

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Amplify the antibody genes from the identified sub-library using tag-specific PCR primers

If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have 1,000 different types of rAbs



Express and purify the antibodies



Re-distribute over an Anti-tag Antibody Array

If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have a single type of rAb



Re-survey to ID the antibody of interest

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ELLER EI HELLER EINCAULIFFE LLP Sheet 17 of 23 FIGURE 14C

summary

master library

			•••	
	Arrays used		1,000	1
1b-libraries	Spot diversity		1,000	1
1,000 si	versity Sub-libraries	1,000		
	Round Library diversity	1,000,000,000	1,000,000	1,000
	Round	0	Ţ	II

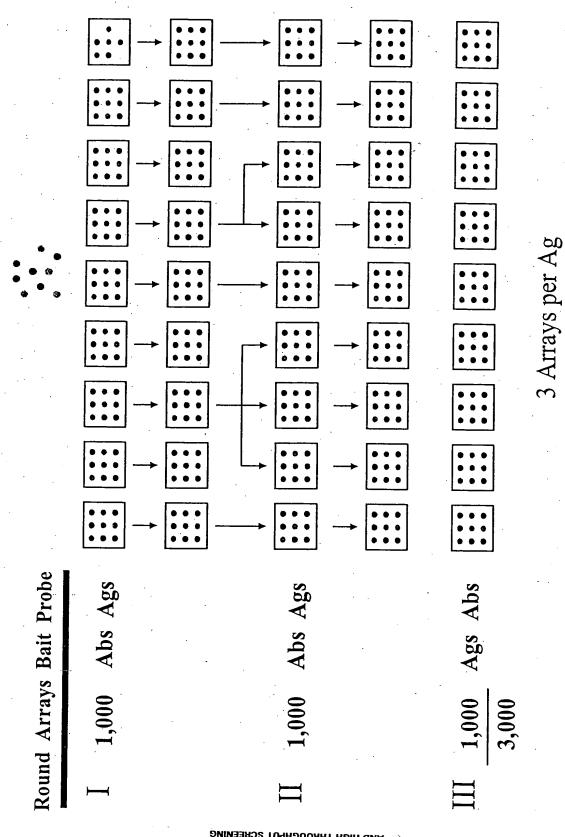
IGURE 14D

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THE STED SORTING
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FIGURE 15

AND HIGH THROUGHPUT SCREENING PROTECTIONS OF BUDDING PROTEINS AND USES THEREOF FOR NESTED SORTING PROTEINS AND USES THE PROTEINS AND USE THE PRO

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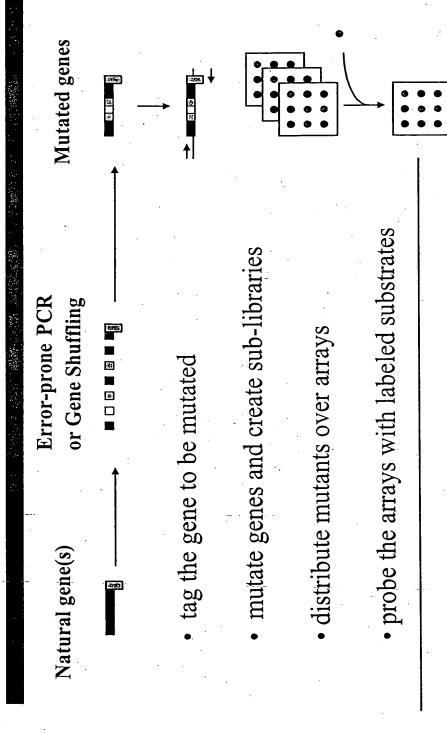
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Enzyme engineering



Spots can contain mixtures of enzymes for detection or pathway engineering

FIGURE 17

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TAGS AND USES THEREOF FOR NESTED SORTING
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THICH THROUGHPUT SCREENING

Protein interaction mapping

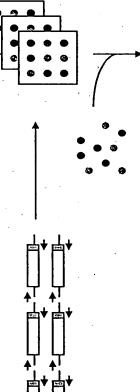
cDNA library

- human tissue
- pathogen

yeast

Generate a tagged cDNA library

Create sub-libraries by PCR



Distribute onto arrays

probe arrays with one or several labeled proteins, peptides, or drugs

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FIGURE 19

AND HIGH THROUGHPUT SCREENING

